



Attorney Docket 27045/1020

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Weinstock and Elliott
Serial No.: 09/362,598
Filed: July 28, 1999
Entitled: USE OF PARASITIC BIOLOGICAL
AGENTS FOR PREVENTION AND
CONTROL OF AUTOIMMUNE
DISEASES

Examiner: R.A. Zeman
Art Unit: 1645
Conf. No.: 7062

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF JOEL V. WEINSTOCK AND DAVID E. ELLIOTT UNDER
37 C.F.R. § 1.132

I, Joel V. Weinstock, hereby declare that:

1. I am one of the inventors in the above-named patent application. I am employed by the University of Iowa as a Physician-Scientist in the Department of Internal Medicine. I received a M.D. from Wayne State University in 1969. I have been employed by the University of Iowa since 1986.

I, David E. Elliott, hereby declare that :

2. I am one of the inventors on the above named patent application. I am employed by the University of Iowa as a Physician-Scientist in the Department of Internal Medicine. I received a Ph.D. in Immunology/Microbiology from Wayne State University in 1985. I received a M.D. from Wayne State University in 1988. I have been employed by the University of Iowa since 1991.

3. The instant application is directed to a method of screening a helminthic parasite preparation for one or more components that reduce a Th1 immune response, the method comprising the steps of: (a) obtaining a helminthic parasite preparation; (b) producing a homogenate of the helminthic parasite preparation; (c) separating fractions of the homogenate; (d) assaying a fraction of the homogenate to determine whether the fraction decreases a Th1

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immune response; wherein a decrease in a Th1 immune response is indicative of the fraction comprising one or more components that reduce the Th1 immune response; and (e) further fractionating the fraction of step (d) into sub-fractions and identifying a sub-fraction that reduces a Th1 immune response.

4. We recognize that iterative fractionation and testing of resulting fractions and sub-fractions for activity, as claimed, is a well-known and routine method for isolating the biologically active component(s) of a complex biological mixture. It is also well known in the art that the same assay can be used at each stage of a fractionation procedure to monitor which fraction(s) or sub-fraction(s) have the activity of interest. For example, when fractionating an enzyme-containing preparation, the same enzyme assay is most often used at each stage of the fractionation procedure to monitor which fraction(s) or sub-fraction(s) contains the activity.

5. The Exhibits attached herewith (Exhibit A-C) further demonstrate that iterative fractionation and testing of resulting fractions and sub-fractions for activity, as claimed, is well-known and routine. Although the ultimate goals of the cited reference were to purify various proteins, all references describe similar fractionation processes. The references clearly establish that the fractionation and testing of resulting fractions and sub-fractions for activity is a well-established practice in the art.

6. In Palczewski et al. (1988, J. Biol. Chem. 263: 14067-14073; Exhibit A), an iterative sub-fractionation approach is used to purify rhodopsin kinase. Although the ultimate goal is to purify rhodopsin kinase to "near homogeneity," a retinal extract was fractionated over a DEAE cellulose column, and all eluted fractions were monitored for rhodopsin kinase activity with a kinase assay (e.g., see Figure 3). As such, Palczewski et al. demonstrate that one skilled in the art would know how to apply the same assay to any fraction of a fractionating procedure, whether the fraction contains rhodopsin kinase or not. In fact, assaying multiple fractions from the fractionating procedure for rhodopsin activity is how one can screen for a fraction that contains rhodopsin kinase to "near homogeneity."

7. Similarly, in Soubeyrand et al. (1997, J. Biol. Chem. 272: 222-227; Exhibit B), an iterative sub-fractionation approach was used to purify a Phospholipase A2 enzyme from seminal plasma. Seminal plasma was fractionated over a butyl-Sepharose column, and a desorbed fraction was applied to a Sephacryl S-300 sieving column, after which fractions were tested for Phospholipase A2 activity. Again, multiple fractions were assayed for Phospholipase A2 activity whether or not the fraction contains Phospholipase A2 (e.g., see Figure 1). The routine activity assay of multiple fractions from the fractionating procedure led to the identification of a purified fraction that contains Phospholipase A2.

8. In Ostergaard et al. (1997, J. Biol. Chem. 272: 30009-30016; Exhibit C), an iterative sub-fractionation approach was used to purify an L-Galactono- γ -Lactone Dehydrogenase (GLDase) from cauliflower plants. A crude cauliflower mitochondrial extract was passed over a DEAE-Sepharose column, and eluted fractions were assayed for GLDase by monitoring the reduction of Cytochrome *c*. Fractions having activity in this assay were passed over a phenyl-Sepharose CL-4B column, and eluted sub-fractions were assayed for GLDase activity using the same assay. The active sub-fractions were pooled and applied to a Sephacryl SF-200 gel filtration column, and the sub-fractions were monitored for activity using the same

GLDase assay. Subsequent sub-fractionation steps included two different anion exchange FPLC sub-fractionations and an HPLC sub-fractionation, with GLDase activity monitoring of the sub-fractions using the same assay at each stage. Again, multiple fractions and sub-fractions, whether containing GLDase or not, are routinely assayed for GLDase activity which led to the purification of GLDase (e.g., see Figure 1).

We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12/8/04
Date

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